

## Beneficial effects of fruit extracts on neuronal function and behavior in a rodent model of accelerated aging

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### Abstract

Exposing young rats to particles of high-energy and charge (HZE particles) enhances indices of oxidative stress and inflammation and disrupts the functioning of the dopaminergic system and behaviors mediated by this system in a manner similar to that seen in aged animals. Previous research has shown that diets supplemented with 2% blueberry or strawberry extracts have the ability to retard and even reverse age-related deficits in behavior and signal transduction in rats, perhaps due to their antioxidant and anti-inflammatory properties. This study evaluated the efficacy of these diets on irradiation-induced deficits in these parameters by maintaining rats on these diets or a control diet for 8 weeks prior to being exposed to whole-body irradiation with 1.5 Gy of 1 GeV/n high-energy <sup>56</sup>Fe particles. Irradiation impaired performance in the Morris water maze and measures of dopamine release 1 month following radiation; these deficits were protected by the antioxidant diets. The strawberry diet offered better protection against spatial deficits in the maze because strawberry-fed animals were better able to retain place information (a hippocampally mediated behavior) compared to controls. The blueberry diet, on the other hand, seemed to improve reversal learning, a behavior more dependent on intact striatal function. These data suggest that <sup>56</sup>Fe particle irradiation causes deficits in behavior and signaling in rats which were ameliorated by an antioxidant diet and that the polyphenols in these fruits might be acting in different brain regions.

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**Keywords:** Morris water maze; Cognitive performance; Irradiation; Oxidative stress; Inflammation; Antioxidants; Dietary supplementation; Polyphenols

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### 1. Introduction

Previous studies have shown that whole-body exposure of rats to HZE particles, primarily 600 MeV or 1 GeV <sup>56</sup>Fe, can produce profound deficits in neuronal functioning and behavior, similar to the adverse changes seen in aged animals [18,19,26]. Exposing rats to HZE particles affects the integrity of the dopaminergic system, possibly due to permanent changes in functioning of dopamine (DA) neurons [19]. Behaviors affected by radiation include deficits

in motor performance [19], spatial learning and memory [5,7,49,50,52], amphetamine-induced conditioned taste aversion learning [35,39,40,43], conditioned place preference [39,41], and operant conditioning (fixed-ratio bar pressing) [33,36]. These deficits occur soon after <sup>56</sup>Fe radiation, have a threshold for effect, lack a dose–response relationship, and fail to show recovery of function following exposure [19,38].

Increased susceptibility to the long-term effects of oxidative stress (OS) and inflammatory insults are thought to be contributing factors to the neurochemical and behavioral deficits seen in normal aging as well as in the progression of neurodegenerative diseases [1,12,13,30]. It is likely

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that the deficits seen from exposure to  $^{56}\text{Fe}$  irradiation also involve insult from increased OS and inflammation on the brain [47,53]. Increases in oxidative stress levels have been observed in the frontal cortex of rats irradiated with 1.5 Gy of  $^{56}\text{Fe}$  particles [7].

Research by our laboratory and that of our collaborators has demonstrated that fruits and vegetables high in antioxidant and anti-inflammatory activity, such as blueberries and strawberries, can prevent the occurrence of the neurochemical and behavioral changes that occur in aging [3,6,11,15,24,55]. Given the similarities between the normal aging process and the effects of exposure to heavy particles [26], it is possible that dietary polyphenolics, which possess antioxidant and anti-inflammatory properties [32,46], will be equally effective in preventing the behavioral changes produced by exposure to  $^{56}\text{Fe}$  particles.

In previous studies, we have shown that maintaining rats on 2% strawberry- or blueberry-supplemented diets prevented the disruption of conditioned taste aversion learning [43], while only the strawberry diet improved deficits in operant responding produced by exposure to 1.5 Gy of  $^{56}\text{Fe}$  particles in rats tested 12 months following irradiation [37]. The strawberry diet also prevented the disruption of operant responding in rats tested 5 and 8 months after exposure to a higher dose of radiation (2.0 Gy of  $^{56}\text{Fe}$  particles); however, when tested 13 and 18 months after irradiation there were no differences in performance between the irradiated rats maintained on control, strawberry or blueberry diets [34]. These observations suggest that the beneficial effects of these diets may be dependent upon the age of testing [34]. Furthermore, animals fed the berryfruit diets prior to exposure showed reduced heavy particle-induced tumorigenesis after 1 year compared to the animals fed the control diet [22]. Interestingly, the animals in these studies were only fed the diet containing strawberries and blueberries for 8 weeks prior to and 1 week following radiation exposure; they were fed an unsupplemented diet after that period.

Taken together, these findings and those previously found in aged animals suggest that foods such as berryfruits may serve as powerful agents in preventing motor and cognitive deficits in aging. The present study was carried out to extend these findings by assessing the ability of 8 weeks of feeding a 2% blueberry- or strawberry-enriched diet to offset the irradiation-induced deficits in spatial learning and memory (as assessed by Morris water maze performance) and signal transduction (oxotremorine-enhanced K(+)-evoked release of dopamine from striatal slices, an indicator of striatal dopamine integrity) produced by exposure to 1.5 Gy of accelerated iron particles. This dose of  $^{56}\text{Fe}$  radiation has been shown to disrupt spatial learning and memory in previous experiments [5,7,50]. By measuring neuronal signaling, specifically muscarinic receptor sensitivity, under basal and conditions of oxidative stress ( $\text{H}_2\text{O}_2$ ), we hope to examine multiple mechanisms that may be involved in the beneficial effects of berryfruits on radiation-induced deficits.

## 2. Materials and methods

### 2.1. Animals

Sixty male Sprague-Dawley rats (Taconic Farms, Germantown, NY), weighing 175–200 g and 2 months of age at the start of the experiment, were used in this study. They were housed in an AAALAC-accredited vivarium in polycarbonate cages at Brookhaven National Laboratory (BNL) in Upton, NY for 2 months prior to irradiation with 1.5 Gy of 1 GeV/n  $^{56}\text{Fe}$  particles. During this time, the rats were given *ad libitum* access to diets containing either 2% blueberry or strawberry extract, or a control diet; twenty rats were fed each diet. At 4 months of age, half of the rats in each diet condition were irradiated while the remaining rats served as non-irradiated controls. One day following radiation, all rats were shipped to the USDA, Human Nutrition Research Center on Aging at Tufts University (HNRCA) in Boston, MA, where they were allowed to acclimate for 4 weeks before behavioral testing. At the HNRCA, the rats were maintained on a 12 h light/dark cycle and were individually housed in hanging wire mesh cages with *ad libitum* access to food and water. These animals were utilized in compliance with all applicable laws and regulations as well as principles expressed in the National Institutes of Health, USPHS, Guide for the Care and Use of Laboratory Animals. This study was approved by the Animal Care and Use Committee of our Center and by those of BNL and the University of Maryland Baltimore County.

### 2.2. Diets

The diets were prepared by homogenizing blueberries or strawberries in water (1:1 or 1:2, w/v, respectively) for 3 min and then centrifuging the recovered homogenate at  $13,000 \times g$  for 15 min at 4 °C. The supernatant was then frozen, crushed, and lyophilized and the freeze dried extracts were shipped to Harlan Teklad (Madison, WI) where they were combined with the control diet, which was a modification of the NIH-31 diet (20 g/kg diet, 2% w/w). This diet is the same one used in previous studies in which beneficial effects on aging were found [6,11,15,55]. The amount of corn in the control diet was adjusted to compensate for the added volume (see Ref. [55] for more information on diet preparation and composition). The rats were maintained on either the control, blueberry, or strawberry diet for 8 weeks prior to irradiation and throughout the rest of the study. At the time of irradiation the rats were divided into six groups ( $n = 10/\text{group}$ ): control diet, non-irradiated; control diet, irradiated; experimental diet (blueberry or strawberry), non-irradiated; experimental diet (blueberry or strawberry), irradiated.

### 2.3. Dosimetry and irradiation procedures

Radiated rats were exposed to whole-body irradiation with high-energy  $^{56}\text{Fe}$  particles (1 GeV/n) at the AGS (Alternating Gradient Synchrotron) at BNL. During exposure to the

particles, the rats were restrained using a well-ventilated plastic tube which was placed perpendicular to the beam and positioned so that the head of the rat was in the center of the beam. An X-ray film was taken to confirm the location of the rat within the beam. The rats were exposed to 1.5 Gy of 1 GeV/n  $^{56}\text{Fe}$  particles at a nominal dose rate of 1.0–1.5 Gy/min. Control rats were not exposed to the beam. The details of the beam and dosimetry have been provided by Zeitlin et al. [56].

#### 2.4. *Morris water maze testing*

The MWM is a commonly used age- [4,14,51], diet- [24,25,55] and radiation-sensitive [50] spatial learning and memory paradigm that requires rats to find the location of a hidden platform (10 cm in diameter) just below the surface (2 cm) of a circular pool of water (134 cm in diameter  $\times$  50 cm in height, maintained at 23 °C) based on distal cues in previous learning trials [29]. Accurate navigation to the platform is rewarded by escape from the water. The maze is placed in a room with the lights dimmed, and there are numerous extramaze cues on the walls that the rat can use to navigate the maze.

Rats were given four consecutive days of training in the MWM (6 trials/day) 4–5 weeks after irradiation. At the beginning of each trial, the rat was gently immersed in the water, facing the wall, at one of three randomized start positions (located in the center of each quadrant not containing the platform). Each rat was allowed 60 s to escape onto the platform; if the rat failed to escape within this time, it was guided to the platform. Once the rat reached the platform, it remained there for 10 s. At the end of each trial, the rat was towel-dried, returned to its home cage for approximately 15–20 min (during which the remaining rats were tested) before being returned to the maze for its next trial. On days 2 and 3, trial 6 was a probe trial (60 s swim), where the platform was removed and the rat's swim path was monitored to measure spatial bias. On day 4 a reversal test (platform moved to the quadrant diagonally opposite to the training quadrant) was performed for 5 trials, followed by a probe trial, in order to assess the ability of the rats to relearn a new platform location. Performances were videotaped and analyzed with image tracking software (HVS Image, UK), which allows measurements of latency to find the platform (s), path length (cm), and swimming speed (cm/s), as well as information on the probe trials, such as number of crossings and time spent in the area where the platform had been previously located. For a more detailed description of the maze and the paradigm used, see Ref. [50].

#### 2.5. *Dopamine release*

Muscarinic enhancement of dopamine (DA) release from superfused striatal slices is an indicator of receptor sensitivity and striatal function and is sensitive to radiation [19,20], aging [16,17,21], and dietary supplementation [24,25,55]. DA release was conducted 2 weeks following behavioral

testing on freshly dissected and cross cut (300  $\mu\text{m}$ ) striatal slices from the brains of animals in the various groups. The slices were placed in small glass vials containing modified Krebs-Ringer basal release medium (BRM) that had been bubbled for 30 min with 95%  $\text{O}_2$ /5%  $\text{CO}_2$  and which contained (in mM)  $\text{NaHCO}_3$  21, glucose 3.4,  $\text{NaH}_2\text{PO}_4$  1.3, EGTA 1,  $\text{MgCl}_2$  0.93,  $\text{NaCl}$  127 and  $\text{KCl}$  2.5 (low  $\text{KCl}$ ) (pH 7.4). Half of the tissue was treated with  $\text{H}_2\text{O}_2$  to assess the effect of radiation and diet under conditions of oxidative stress (OS). The slices were then placed in the perfusion chambers where they were maintained at 37 °C and perfused with the BRM for 30 min. Following this equilibration period, the medium was then switched to one containing (in mM)  $\text{KCl}$  30,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  1.26 (in place of EGTA) and  $\text{NaCl}$  57, and 0 or 500  $\mu\text{M}$  oxotremorine, and the enhancement of potassium-evoked dopamine release was assessed. DA release was quantitated by HPLC coupled to electrochemical detection and expressed as pmol/mg protein as determined by the Lowry and colleagues procedure [27].

#### 2.6. *Statistical analyses*

For each measure, between-subjects analysis of variance (ANOVA) models with two grouping factors (radiation and diet) were performed using Systat (SPSS Inc., Chicago, IL) to test for statistical significance at the  $p < 0.05$  level. For the MWM data, days or trials, when appropriate, were included in the model as a within-subjects variable (repeated measures analysis). Because we hypothesized that radiation would have differential effects depending on diet, the variable of interest was the three-way interaction of radiation  $\times$  diet  $\times$  days/trials. Post-hoc comparisons, to determine differences among the groups, were performed using Fisher's LSD post-hoc analysis on each day individually. The data for the non-irradiated blueberry and strawberry groups are not included in the figures because they did not differ from the control diet-fed non-irradiated animals, and not including them made it easier to visualize the changes due to radiation and radiation plus diet.

MWM dependent measures included escape latency and swim speed. On the probe trials, dependent measures also included percent time spent searching in each of the four quadrants of the pool as well as number of crossings of, latency to, and percent time in the region of the pool marking the exact position and surface area of the previous location of the hidden platform.

### 3. Results

#### 3.1. *Morris water maze performance*

Radiation exposure impaired cognitive behavior, particularly on the probe trials and on the reversal day; the strawberry and blueberry diets were able to protect against these harmful radiation effects. Overall, when examining learning,

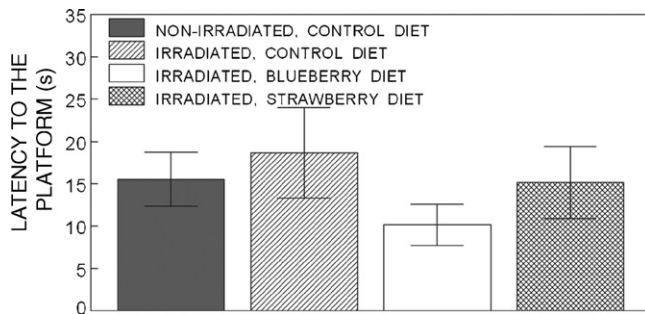


Fig. 1. Escape latencies (seconds, mean  $\pm$  S.E.M.) to find the hidden platform in the Morris water maze for reversal testing on day 4 (trials 2–5) for non-irradiated rats, and irradiated rats fed the control, blueberry, and strawberry diets.

measured by latency to find the platform on days 1–3 when the rats were still learning the task, there was a significant effect of radiation [ $F(1, 54) = 9.16, p < 0.01$ ] and testing day [ $F(2, 108) = 107.39, p < 0.001$ ], but no overall diet effect. However, there was a significant effect of diet as a function of radiation and trials [ $F(8, 216) = 2.24, p < 0.05$ ], i.e., the diets showed differential effects with radiation over the trials.

### 3.1.1. Reversal trials

On day 4, when the position of the platform was changed to a different quadrant, the irradiated, control diet animals tended to have a longer latency to the platform than the irradiated, blueberry diet animals, but this difference did not reach statistical significance (Fig. 1).

### 3.1.2. Probe trials

The irradiated control diet rats had a longer latency to cross the previous platform location than the non-irradiated controls on the probe trials as a function of day of testing [radiation  $\times$  day effect,  $F(2, 108) = 4.09, p < 0.05$ ] (Fig. 2). Post-hoc comparisons of the responses of the irradiated and control groups showed that this difference was significant on day 3 ( $p < 0.05$ ). Additionally, there was

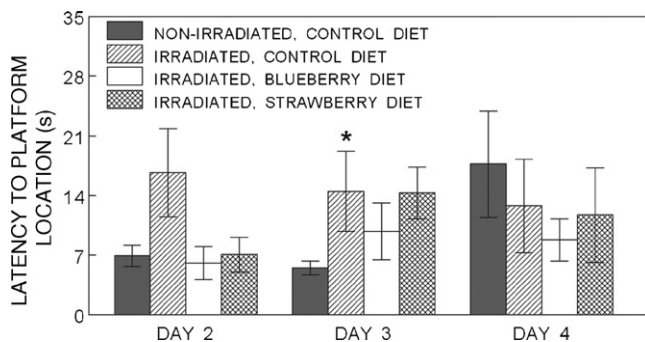


Fig. 2. Latency to cross (seconds, mean  $\pm$  S.E.M.) the previous location of the hidden platform for the first time during the probe trials on testing days 2–4 in the Morris water maze for non-irradiated rats, and irradiated rats fed the control, blueberry, and strawberry diets. An asterisk indicates a significant difference between the non-irradiated and irradiated control fed groups on that day ( $p < 0.05$ ).

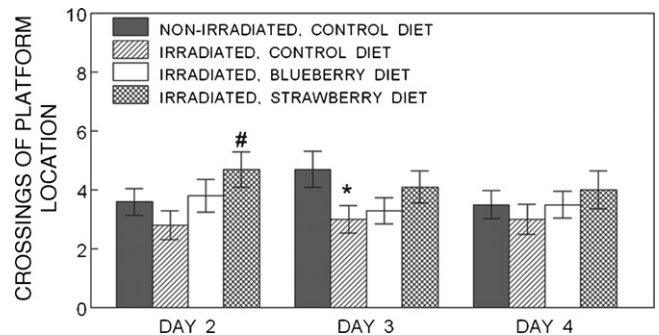


Fig. 3. Number of crossings (mean  $\pm$  S.E.M.) of the previous location of the hidden platform during the probe trials on testing days 2–4 in the Morris water maze for non-irradiated rats, and irradiated rats fed the control, blueberry, and strawberry diets. An asterisk indicates a significant difference between the non-irradiated and irradiated control fed groups on that day ( $p < 0.05$ ), while the number sign indicates a significant difference from the irradiated control diet group on that day ( $p < 0.05$ ).

also an effect of diet as a function of radiation and day of testing [radiation  $\times$  diet  $\times$  day effect,  $F(4, 108) = 3.12, p < 0.05$ ]. It appeared that blueberry supplementation may have protected against the radiation-induced deficits, particularly on day 2; however, this effect did not reach significance.

Also during the probe trials, the irradiated controls had significantly fewer crossings of the previous platform location than the non-irradiated controls as a function of the day of testing [radiation  $\times$  day effect,  $F(2, 108) = 3.63, p < 0.05$ ], particularly on day 3 ( $p < 0.05$ ) (Fig. 3). However, these analyses also revealed that there was a significant effect of diet on the irradiated animals [radiation  $\times$  diet effect,  $F(2, 54) = 5.61, p < 0.01$ ], such that the irradiated strawberry animals exhibited a greater number of crossings of the former platform location than the irradiated controls on day 2 ( $p < 0.05$ ).

There was also an effect of the strawberry diet in the irradiated animals in percent time spent in the platform quadrant on the probe trials [radiation  $\times$  diet effect,  $F(2, 54) = 3.98, p < 0.05$ ] (Fig. 4), where post-hoc analyses revealed that these

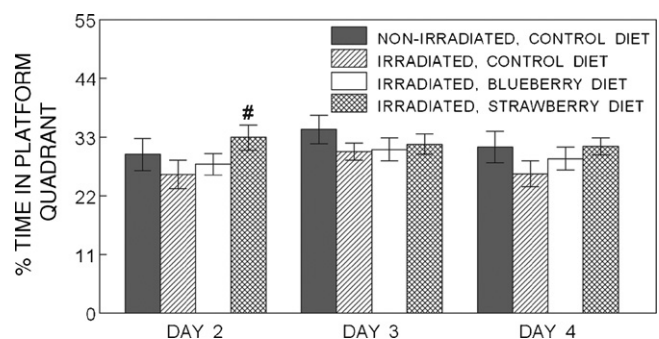


Fig. 4. Percent time (mean  $\pm$  S.E.M.) spent in the quadrant that had previously contained the platform during the probe trials on testing days 2–4 in the Morris water maze for non-irradiated rats, and irradiated rats fed the control, blueberry, and strawberry diets. The number sign indicates a significant difference from the irradiated control diet group on that day ( $p < 0.05$ ).



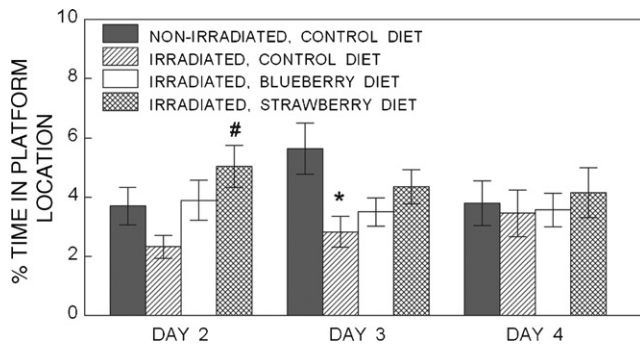


Fig. 5. Percent time (mean  $\pm$  S.E.M.) spent in the previous location of the hidden platform during the probe trials on testing days 2–4 in the Morris water maze for non-irradiated rats, and irradiated rats fed the control, blueberry, and strawberry diets. An asterisk indicates a significant difference between the non-irradiated and irradiated control fed groups on that day (\* $p < 0.05$ ), while the number sign indicates a significant difference from the irradiated control diet group on that day (# $p < 0.05$ ).

animals spent significantly more time in the platform quadrant than the irradiated rats fed the control diet rats on day 2 ( $p < 0.05$ ).

The irradiated, control diet animals spent significantly less time in the platform location than the non-radiated controls [radiation  $\times$  day effect,  $F(2, 108) = 4.51$ ,  $p < 0.05$ ], particularly on day 3 ( $p < 0.01$ ) (Fig. 5). There was also a significant radiation  $\times$  diet effect [ $F(2, 54) = 4.23$ ,  $p < 0.05$ ], as the diets protected against these radiation-induced deficits on day 2. Compared to irradiated rats fed the control diet, the irradiated, blueberry diet animals tended to spend more time in the platform location ( $p = 0.06$ ), while the irradiated, strawberry diet animals spent significantly more time in the platform location ( $p < 0.01$ ).

The differences in performance between the irradiated and control rats were not due to swim speed, as there were no significant effects of radiation on this parameter ( $p > 0.05$ ). There were also no differences in body weight, measured during MWM testing, due to radiation or diet group ( $p > 0.05$ ).

### 3.2. DA release

There were significant effects of diet on DA release under basal ( $-H_2O_2$ ) conditions [ $F(2, 24) = 23.62$ ,  $p < 0.01$ ], that were also a function of the radiation condition [ $F(2, 24) = 24.19$ ,  $p < 0.01$ ] (Fig. 6A). The irradiated controls had less DA release than the non-irradiated controls ( $p < 0.01$ ), however this deficit was prevented by both the blueberry ( $p < 0.01$ ) and strawberry diet ( $p < 0.01$ ). Similarly, under OS conditions ( $+H_2O_2$ ), there was a significant effect of diet [ $F(2, 24) = 13.93$ ,  $p < 0.01$ ], with the diet effect under the particular radiation condition approaching significance [ $F(2, 24) = 2.77$ ,  $p = 0.08$ ] (Fig. 6B). However, both the blueberry ( $p < 0.01$ ) and strawberry ( $p < 0.01$ ) diets significantly protected against the effects of radiation on DA release compared to irradiated animals on the control diet.

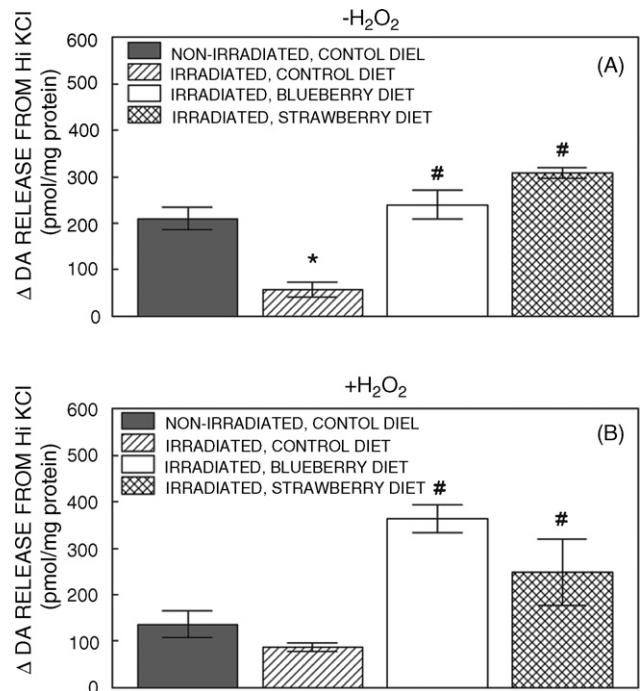


Fig. 6. Dopamine (DA) release (change in pmol/mg protein) in striatal tissues of non-irradiated rats, and irradiated rats fed the control, blueberry, and strawberry diets under basal levels ( $-H_2O_2$ ; A) and conditions of oxidative stress ( $+H_2O_2$ ; B). An asterisk indicates a significant difference between the non-irradiated and irradiated control fed groups (\* $p < 0.05$ ), while the number sign indicates a significant difference from the irradiated control diet group (# $p < 0.05$ ).

## 4. Discussion

This study showed that  $^{56}Fe$  particle irradiation causes deficits in behavior, as measured by the MWM, and signaling, as measured by DA release, in rats and further demonstrate that radiation causes cognitive and neuronal changes in rats similar to those seen in aged animals [18,19,26]. The effects of radiation were primarily seen on day 3 during the probe trials, perhaps due to increased learning in the non-irradiated control diet group from days 2 to 3, while the irradiated, control fed animals did not improve, i.e., did not learn spatial strategies to solve the maze or did not remember what they had learned from previous trials. Aged rats also show a lack of spatial preference compared to young animals when tested in a probe trial (i.e., less time spent in the training quadrant and less platform crossings) [9,44]. Interestingly, there were no detrimental effects of radiation on day 4 when the platform was moved to a new location, although we have previously found deficits in day 4 relearning in rats irradiated with the same dose and particle type used in the present study [50]. Aged animals also have difficulty learning a new platform location during reversal training [10].

These data also illustrate that the blueberry and strawberry diets, high in antioxidants, provide protection against harmful radiation effects, specifically in spatial learning and memory and in striatal dopamine release under basal and

OS conditions. Furthermore, it appeared that the antioxidant diets and the different polyphenols in these fruits might be acting in different brain regions to produce their beneficial effects. The irradiated, blueberry diet animals tended to have improved performance in latency measures (i.e., shorter latencies to the platform on day 4 when the position changed and shorter latencies to cross the platform location on the probe trials), which involves reversal learning dependent on intact striatal functioning [28]. Conversely, the irradiated, strawberry diet animals showed fewer deficits on the probe trial measures in that they spent more time in the platform quadrant and location and had a greater number of crossings of the platform location on probe trial days compared to irradiated controls, suggesting retained place information, which is a hippocampally dependent behavior [8,28,31]. Perhaps, the polyphenolic compounds in blueberries are working mainly in the striatum, while those in strawberries are primarily affecting the hippocampus. While these results are interesting, more testing needs to be done to confirm this suggestion.

Berries contain bioactive phytochemicals that have been identified as flavonoids (anthocyanins, flavonols and flavanols), condensed tannins (proanthocyanidins), hydrolysable tannins (ellagitannins and gallotannins), stilbenoids, phenolic acids (hydroxybenzoic and hydroxycinnamic acids) and lignans. Specific berries may contain an abundance of a particular group of phytochemicals. For example, among common edible berries, blueberries and cranberries contain high levels of proanthocyanidins, while strawberries, raspberries, blackberries and black raspberries contain high levels of ellagitannins. Anthocyanins are ubiquitous to all berries accounting for their brilliant colors [48]. The relative amounts of these compounds in different fruits vary, which may account for differences in antioxidant capacity as well as effectiveness. Additionally, the determination as to which flavonoids can cross the blood brain barrier depends on their properties, such as charged state, lipophilicity, and interactions with efflux transporters, with possible relative specificity of the polyphenols for different brain areas (for review, see Ref. [54]). As mentioned above, based on the MWM data, it may be that the greatest efficacy of compounds such as the proanthocyanidins may be found in the striatum while that of the strawberry polyphenols (e.g., ellagitannins) may involve the hippocampus.

One reason for the similarity in behavior deficits between aged and irradiated rats might be an increase in oxidative stress or inflammation, which may be responsible for the induction of both radiation- and age-related cognitive deficits. Our research has shown that supplementation with fruits and vegetables is beneficial in both forestalling and reversing the deleterious effects of aging on neuronal communication and behavior (reviewed in [23]). Furthermore, we have shown that maintaining rats on strawberry- or blueberry-supplemented diets prior to radiation exposure prevented the disruption of conditioned taste aversion learning [43] and operant responding [34,37]. Animals fed the berryfruit diets prior to exposure also showed reduced heavy particle-induced tumorigenesis

after 1 year compared to the animals fed the control diet [22]. Therefore, the observed protection seen in our aging and radiation models may be the result of the antioxidant and anti-inflammatory properties of the polyphenolic compounds found in these fruits and vegetables [45].

As seen in our previous research [18,19,20,26], one aspect of neuronal signaling (i.e., the sensitivity of the muscarinic receptors) as measured by DA release, was adversely affected by radiation under basal conditions. Interestingly, under conditions of oxidative stress, this difference did not reach significance, possibly because of a floor effect. The addition of  $H_2O_2$  to the tissue decreased DA release in the non-irradiated control fed diets to such low levels that radiation was not able to decrease levels much below this point. Numerous studies have shown that the loss of muscarinic receptor sensitivity in aging is the result of decrements in receptor-G protein uncoupling/coupling that do not allow the neuronal signal to be transmitted further down the pathway to mobilize calcium upon depolarization [16,17,20,21]. Studies have shown that these decrements are also seen in aged animals and may lead to both cognitive and motor deficits [16,17,21]. Both the blueberry and the strawberry diets were able to protect against these decreases in DA release, under both basal and OS conditions. These results indicate that not only are the polyphenols in these fruits at least partially working through antioxidant mechanisms, but possibly through other actions that do not involve oxidative stress, since the berryfruits were also protective under basal conditions. Our laboratory has previously shown that blueberry supplementation increases hippocampal plasticity and cognitive performance in aged rats via concerted putative mechanisms involving neurogenesis, neurotrophic factor IGF-1 and its receptor, and MAP kinase signal transduction cascades [6]. Furthermore, we have shown that APP/PS1 (i.e., amyloid precursor protein/presenilin-1, mutations) transgenic mice (used as a murine model for Alzheimer's Disease) fed a blueberry diet did not have deficits in behavior compared to transgenic non-supplemented mice, possibly due to increased levels of extracellular signal regulated kinase (ERK) and protein kinase C (PKC) [15]. However, no difference in the number of plaques was observed between the two groups. Therefore, berryfruit supplementation might enhance neuronal signaling and function to offset the putative deleterious effect of plaque deposition on behavioral deficits seen in the transgenic mice [15] as well as in normal aging [6].

These findings suggest that multiple mechanisms may be involved in the beneficial effects of high antioxidant fruits on aging as well as radiation. They further suggest that radiation from HZE particles may produce behavioral and neuronal effects that parallel those seen in aging. The decrements seen in behavior following low doses of HZE particles may impair the ability of astronauts to perform critical tasks on long-duration missions outside the magnetic field of the Earth, such as a mission to Mars. During these missions, astronauts will be exposed to doses and types of radiation that differ from those experienced in Low Earth Orbit, where the International

Space Station and Space Shuttle operate [2], and exposure of astronauts to these radiation sources may affect their ability to successfully complete mission requirements. Therefore, it may be important to include berryfruit or berryfruit extract supplementation as countermeasures to mitigate the effects of radiation during long-term missions in space [42] and also to reduce or reverse the deleterious effects of aging.

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## References

- [1] Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci USA* 1993;90:7915–22.
- [2] Badhwar GD. The radiation environment in low earth orbit. *Radiat Res* 1997;148:S3–S10.
- [3] Bickford P, Gould T, Briederick L, Chadman K, Pollock A, Young D, et al. Antioxidant-rich diets improve cerebellar physiology and motor learning in aged rats. *Brain Res* 2000;866:211–7.
- [4] Brandeis R, Brandys Y, Yehuda S. The use of the Morris Water Maze in the study of memory and learning. *Int J Neurosci* 1989;48:29–69.
- [5] Casadesus G, Shukitt-Hale B, Cantuti-Castelvetri I, Rabin BM, Joseph JA. The effects of heavy particle irradiation on exploration and response to environmental change. *Adv Space Res* 2004;33:1340–6.
- [6] Casadesus G, Shukitt-Hale B, Stellwagen HM, Zhu X, Lee H-G, Smith MA, et al. Modulation of hippocampal plasticity and cognitive behavior by short-term blueberry supplementation in aged rats. *Nutr Neurosci* 2004;7:309–16.
- [7] Denisova NA, Shukitt-Hale B, Rabin BM, Joseph JA. Brain signaling and behavioral responses induced by exposure to  $^{56}\text{Fe}$ -particle radiation. *Rad Res* 2002;158:725–34.
- [8] Devan BD, Goad EH, Petri HL. Dissociation of hippocampal and striatal contributions to spatial navigation in the water maze. *Neurobiol Learn Mem* 1996;66:305–23.
- [9] Frick KM, Baxter MG, Markowska AL, Olton DS, Price DL. Age-related spatial reference and working memory deficits assessed in the water maze. *Neurobiol Aging* 1995;16:149–60.
- [10] Gage FH, Dunnett SB, Bjorklund A. Spatial learning and motor deficits in aged rats. *Neurobiol Aging* 1984;5:43–8.
- [11] Goyarzu P, Malin DH, Lau FC, Tagliatalata G, Moon WD, Jennings R, et al. Blueberry supplemented diet: effects on object recognition memory and Nuclear Factor-Kappa B levels in aged rats. *Nutr Neurosci* 2004;7:75–83.
- [12] Harman D. Role of free radicals in aging and disease. *Ann NY Acad Sci* 1992;673:126–41.
- [13] Hauss-Wegrzyniak B, Vannucchi MG, Wenk GL. Behavioral and ultrastructural changes induced by chronic neuroinflammation in young rats. *Brain Res* 2000;859:157–66.
- [14] Ingram DK, Jucker M, Spangler E. Behavioral manifestations of aging. In: Mohr U, Cunnworth DL, Capen CC, editors. *Pathobiology of the aging rat*, vol. 2. Washington: ILSI Press; 1994. p. 49–70.
- [15] Joseph JA, Arendash G, Gordon M, Diamond D, Shukitt-Hale B, Morgan D. Blueberry supplementation enhances signaling and prevents behavioral deficits in an Alzheimer disease model. *Nutr Neurosci* 2003;6:153–62.
- [16] Joseph JA, Dalton TK, Hunt WA. Age-related decrements in the muscarinic enhancement of  $\text{K}^+$ -evoked release of endogenous striatal dopamine: an indicator of altered cholinergic-dopaminergic reciprocal inhibitory control in senescence. *Brain Res* 1988;454:140–8.
- [17] Joseph JA, Dalton TK, Roth GS, Hunt WA. Alterations in muscarinic control of striatal dopamine autoreceptors in senescence: a deficit at the ligand-muscarinic receptor interface? *Brain Res* 1988;454:149–55.
- [18] Joseph JA, Erat S, Rabin BM. CNS effects of heavy particle irradiation in space: behavioral implications. *Adv Space Res* 1998;22:209–16.
- [19] Joseph JA, Hunt WA, Rabin BM, Dalton TK. Possible “accelerated striatal aging” induced by  $^{56}\text{Fe}$  heavy-particle irradiation: implications for manned space flights. *Radiat Res* 1992;130:88–93.
- [20] Joseph JA, Hunt WA, Rabin BM, Dalton TK, Harris AH. Deficits in the sensitivity of striatal muscarinic receptors induced by  $^{56}\text{Fe}$  heavy-particle irradiation: further “age-radiation” parallels. *Radiat Res* 1993;135:257–61.
- [21] Joseph JA, Kowatch MA, Maki T, Roth GS. Selective cross activation/inhibition of second messenger systems and the reduction of age-related deficits in the muscarinic control of dopamine release from perfused rat striata. *Brain Res* 1990;537:40–8.
- [22] Joseph JA, Shukitt-Hale B, Carey A, Rabin BM. Strawberry or blueberry supplementation may protect against tumorigenesis following heavy particle irradiation. *Adv Space Res*, in press.
- [23] Joseph JA, Shukitt-Hale B, Casadesus G. Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am J Clin Nutr* 2005;81:313S–6S.
- [24] Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ, et al. Reversals of age-related declines in neuronal signal transduction cognitive and motor behavioral deficits with blueberry spinach or strawberry dietary supplementation. *J Neurosci* 1999;19:8114–21.
- [25] Joseph JA, Shukitt-Hale B, Denisova NA, Prior RL, Cao G, Martin A, et al. Long term dietary strawberry, spinach, or vitamin E supplementation retards the onset of age related neuronal signal-transduction and cognitive behavioral deficits. *J Neurosci* 1998;18:8047–55.
- [26] Joseph JA, Shukitt-Hale B, McEwen J, Rabin BM. CNS-induced deficits of heavy particle irradiation in space: the aging connection. *Adv Space Res* 2000;25:2057–64.
- [27] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:256–75.
- [28] McDonald RJ, White NM. Parallel information processing in the water maze: evidence for independent memory systems involving dorsal striatum and hippocampus. *Behav Neural Biol* 1994;61:260–70.
- [29] Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Meth* 1984;11:47–60.
- [30] Olanow CW. A radical hypothesis for neurodegeneration. *Trends Neurosci* 1993;16:439–44.
- [31] Oliveira MGM, Bueno OFA, Pomarico AC, Gugliano EB. Strategies used by hippocampal- and caudate-putamen-lesioned rats in a learning task. *Neurobiol Learn Mem* 1997;68:32–41.
- [32] Olszanecki R, Gebeska A, Kozlovski VI, Gryglewski RJ. Flavonoids and nitric oxide synthase. *J Physiol Pharmacol* 2002;53:571–84.
- [33] Rabin BM, Buhler LL, Joseph JA, Shukitt-Hale B, Jenkins DG. Effects of exposure to  $^{56}\text{Fe}$  particles or protons on fixed-ratio operant responding in rats. *J Radiat Res* 2002;43(Suppl.):S225–8.
- [34] Rabin BM, Carrihill-Knoll KL, Carey A, Shukitt-Hale B, Joseph JA. Effect of diet on the disruption of operant responding at different ages following exposure to  $^{56}\text{Fe}$  particles. *Age* 2005;27:69–73.

- [35] Rabin BM, Joseph JA, Erat S. Effects of exposure to different types of radiation on behaviors mediated by peripheral or central systems. *Adv Space Res* 1998;22:217–25.
- [36] Rabin BM, Joseph JA, Shukitt-Hale B. A longitudinal study of operant responding in rats irradiated when two months old. *Radiat Res* 2005;164:552–5.
- [37] Rabin BM, Joseph JA, Shukitt-Hale B. Effects of age and diet on the heavy particle-induced disruption of operant responding produced by a ground-based model for exposure to cosmic rays. *Brain Res* 2005;1036:122–9.
- [38] Rabin BM, Joseph JA, Shukitt-Hale B. Heavy particle irradiation, neurochemistry and behavior: thresholds, dose-response curves and recovery of function. *Adv Space Res* 2004;33:1330–3.
- [39] Rabin BM, Joseph JA, Shukitt-Hale B. Long-term changes in amphetamine-induced reinforcement and aversion in rats following exposure to  $^{56}\text{Fe}$  particles. *Adv Space Res* 2003;31:127–33.
- [40] Rabin BM, Joseph JA, Shukitt-Hale B, McEwen J. Effects of exposure to heavy particles on a behavior mediated by the dopaminergic system. *Adv Space Res* 2000;25:2065–74.
- [41] Rabin BM, Shukitt-Hale B, Joseph JA, Denisova N. Effects of exposure to  $^{56}\text{Fe}$  particles on the acquisition of a conditioned place preference in rats. *Phys Med* 2001;27(Suppl. 1):196–7.
- [42] Rabin BM, Shukitt-Hale B, Joseph JA, Todd P. Diet as a factor in behavioral radiation protection following exposure to heavy particles. *Gravitat Space Biol* 2005;18:11–7.
- [43] Rabin BM, Shukitt-Hale B, Szprengiel B, Joseph JA. Effects of heavy particle irradiation and diet on amphetamine- and lithium chloride-induced taste aversion learning in rats. *Brain Res* 2002;953:31–6.
- [44] Rapp PR, Rosenberg RA, Gallagher M. An evaluation of spatial information processing in aged rats. *Behav Neurosci* 1987;101:3–12.
- [45] Rice-Evans CA, Miller NJ. Antioxidant activities of flavonoids as bioactive components of food. *Biochem Soc Trans* 1996;24:790–4.
- [46] Rice-Evans CA, Miller MJ, Bolwell PG, Branley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolics flavonoids. *Free Radic Res* 1995;22:375–83.
- [47] Riley PA. Free radicals in biology: oxidative stress and the effects of ionizing radiation. *Int J Radiat Biol* 1994;65:27–33.
- [48] Seeram NP. Berries and cancer. In: Heber D, Blackburn GL, Go VLW, editors. *Nutritional oncology*. London, UK: Academic Press; 2005.
- [49] Shukitt-Hale B, Casadesus G, Cantuti-Castelvetri I, Rabin BM, Joseph JA. Cognitive deficits induced by  $^{56}\text{Fe}$  radiation exposure. *Adv Space Res* 2003;31:119–26.
- [50] Shukitt-Hale B, Casadesus G, McEwen JJ, Rabin BM, Joseph JA. Spatial learning and memory deficits induced by exposure to iron-56-particle radiation. *Rad Res* 2000;154:28–33.
- [51] Shukitt-Hale B, Mouzakis G, Joseph JA. Psychomotor and spatial memory performance in aging male Fischer 344 rats. *Exp Gerontol* 1998;33:615–24.
- [52] Shukitt-Hale B, Szprengiel A, Pluhar J, Rabin BM, Joseph JA. The effects of proton exposure on neurochemistry and behavior. *Adv Space Res* 2004;33:1334–9.
- [53] Sun J, Chen Y, Li M, Ge Z. Role of antioxidant enzymes on ionizing radiation resistance. *Free Rad Biol Med* 1998;24:586–93.
- [54] Youdim KA, Shukitt-Hale B, Joseph JA. Flavonoids and the brain: interactions at the blood brain barrier and their physiological effects on the central nervous system. *Free Rad Biol Med* 2004;37:1683–93.
- [55] Youdim KA, Shukitt-Hale B, Martin A, Wang H, Denisova N, Bickford PC, et al. Short term dietary supplementation of blueberry polyphenolics: beneficial effects on aging brain performance and peripheral tissue function. *Nutr Neurosci* 2000;3:383–97.
- [56] Zeitlin C, Heilbronn L, Miller J. Detailed characterization of the 1087 MeV/nucleon iron-56 beam used for radiobiology at the alternating gradient synchrotron. *Radiat Res* 1998;149:560–9.